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91. Hunting for *Phytophthora ramorum* and other species of *Phytophthora* in suburban waterways in South Carolina. Wamishe, Y. A., Jeffers, S. N., and Hwang, J. Phytopathology 97(7)Suppl:S119. 2007.

have affected maize symbionts' evolutionary history. Viral transmission takes place through anastomosis of *U. maydis* cells; thus, the population dynamics of the hosts may strongly affect the population structure of the virus. High viral mutation rates allow us to infer the evolution and divergence of Umv-H1 lineages resulting from changes in host geographic and genetic structure. We determined the phylogeographic history and genetic structure of viral populations in the Americas using analyses of viral nucleotide sequence. We, also, assessed infection frequencies, genetic diversity, rates of neutral evolution, and migration to infer the underlying evolutionary processes affecting ancestral and descendent populations. Results show that viral sequences cluster according to geography and there is a non-random distribution of viral genotypes across the Americas. Evidence of rare long-distance migration of the fungus and virus was detected. Considering a hypothesis of a Mexican geographic origin, it was unexpected that we found populations in South America to have the highest level of genetic diversity. However, this increase in genetic diversity may also be caused by differences in selection, mutation rate heterogeneity, and homoplasy.

Differential gene expression pre- and post-sporulation in the grapevine powdery mildew pathogen

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In grapevine powdery mildew (*Uncinula necator* syn. *Erysiphe necator*) continual production of conidia is a driving force of epidemics. Thus, suppressing sporulation is a logical avenue for control. We have begun to identify and characterize genes differentially expressed throughout the sporulation process beginning with the initiation of sporulation in nascent colonies through the natural suppression of sporulation during ascocarp initiation. To assay gene expression encompassing these developmental stages, RNA was extracted from colonies at five time points: immediately before initiation of conidiation, at conidiophore initiation, during production of conidia, and immediately before and immediately after initiation of ascocarps. Differential gene expression across all time points was analyzed using cDNA-AFLP. Preliminary analysis of differential transcripts suggests that genetic control of sporulation in *U. necator* has some correlation to genes identified in other systems but possibly contains unique elements as well.

Root colonization of several species of native grasses by *Ophiosphaerella herpotricha*

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Spring dead spot, caused by *O. herpotricha*, is the most damaging disease of turf-type bermudagrass (*Cynodon* spp.) in Oklahoma. The objective of this study was to evaluate root colonization of native grasses in the greenhouse by an isolate of *O. herpotricha* obtained from bermudagrass. Soil in pots containing established native grasses (*Buchloe dactyloides*, *Chloris cucullatta*, *Eragrostis secundiflora*, *Eragrostis trichodes*, *Sporabolus airoides*, and *Tridens strictus*) was inoculated with wheat grain infested with *O. herpotricha*. Non-inoculated pots containing the same plant species served as a control. Fourteen months after inoculation, plants appeared healthy and a sub-sample of roots from all plants was assayed on potato dextrose agar for the presence of *O. herpotricha*. The fungus was recovered from at least one plant of each species. Recovery ranged from 11 to 25% of the pots inoculated. The results of this study suggest that *O. herpotricha* may survive in the roots of native grasses for extended periods under greenhouse conditions.

Tissue repair in fusiform rust-infected loblolly and slash pines

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Healing of wounds caused by *Cronartium quercuum* f. sp. *fusiforme* is a complex process that influences the cambial tissues of the pine host. These tissues inhibit the capacity of the fungus to invade stems of loblolly and slash pines. This disruption of the cambium can result in death of stem tissues including resin canals. Herein, we describe events of tissue repair and regression of gall growth on loblolly and slash pines. We used light microscopy to measure changes in cellular traits in restoring cambial and cortical tissues to their normal state. Over 25% of the nuclei in affected clusters of cortical cells stained abnormally and the cells died. Callus cells grew inward and replaced the remaining dead tissue. Haustoria became necrotic in the clusters of dying cells. The number of tannin cells in the cortex averaged 24%. Tissue healing was evident in the formation of reaction zones and callus growth. This activity was stabilized by the accumulation of secondary cell products such as phenols. The resilience of the healing process was further evident by the selective death of branch galls that grew to the stem.

Hunting for *Phytophthora ramorum* and other species of *Phytophthora* in suburban waterways in South Carolina

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In 2004, container-grown nursery plants contaminated with *Phytophthora ramorum* were shipped from several nurseries in California and Oregon to nurseries around the USA. To determine if *P. ramorum* escaped from these plants and became established in local ecosystems, waterways are being monitored in South Carolina cities where nurseries that received contaminated plants are located. At the same time, the prevalence and diversity of other species of *Phytophthora* are being investigated. Streams that drain large suburban landscape areas were targeted. Water samples (1 to 2 liters) were collected from 20 suburban streams in five cities in spring and fall 2006; three to seven streams were sampled in each city. For each water sample, eight aliquots (50 to 250 ml, depending on water quality) were passed through membrane filters (Nuclepore with 3-µm pores or Durapore with 5-µm pores) to trap propagules of *Phytophthora* spp., and filters were inverted on PARPH-V8 selective medium. To date, *P. ramorum* has not been detected in any stream; however, *Phytophthora* spp. were recovered from all 20 suburban streams and the diversity of species appeared to be greater in fall than in spring. Identification of these species is in progress; to date, *P. gonapodyides* has been confirmed in all 20 streams. Monitoring of suburban streams will continue in 2007.

Optimizing *Lettuce infectious yellows virus* cDNA inoculation systems for protoplast and plant infection

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Lettuce infectious yellows virus (LIYV) has a bipartite, positive-sense ssRNA genome and is the type member of the genus *Crinivirus* in the family *Closteroviridae*. LIYV is the only *Crinivirus* for which infectious clones of genomic RNAs have been developed so far. LIYV cDNA-derived *in vitro* transcripts are infectious for *Nicotiana benthamiana* and *N. tabacum* protoplasts, and LIYV can be transmitted back to plants after feeding virions to the whitefly, *Bemisia tabaci*. However, improvement is needed to increase inoculation efficiency and simplicity. We constructed binary plasmid 35S-driven constructs and attempted to inoculate plants via agro-infiltration. No LIYV infection was observed from agro-infiltration using the two original 35S-driven constructs. Further attempts including co-infiltrations with different gene silencing suppressors and modifying the predicted RNA 1 5′ nucleotide sequence were done, however no whole plant infections were obtained. We also used two different ribozymes predicted to give different RNA 1 3′ termini in order to see if this might affect RNA 1 replication ability. The *Hepatitis delta virus* (HDV) ribozyme proved to be effective for yielding infectious LIYV RNA 1 transcripts in protoplasts, as judged by GFP expression of a LIYV RNA 2 defective RNA when it was co-inoculated with RNA 1. We are now constructing additional 35S-driven RNA 1 clones that contain the HDV ribozyme in attempts to develop a direct whole plant infection method.

A high throughput screen using virus-induced gene silencing in *Nicotiana benthamiana* identifies the requirement of squalene synthase for nonhost resistance

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Nonhost resistance is the most common form of disease resistance exhibited by plants against the majority of potential pathogens in nature. We used virus-induced gene silencing (VIGS) in *Nicotiana benthamiana* to identify genes involved in nonhost resistance. We individually silenced 3,000 genes by using cDNA clones from a normalized NbcDNA library. Eleven genes were identified to be involved in type I and/or type II nonhost resistances. One of them encoding squalene synthase (SQS), a key enzyme catalyzing the first enzymatic step in sterol biosynthesis, was further characterized by RNA interference (RNAi) and gene overexpression in both *N. benthamiana* and *Arabidopsis*. The transgenic *SQS* RNAi lines of *Arabidopsis* were not only susceptible to nonhost pathogens, *Pseudomonas syringae* pv. *tabaci* and *P. syringae* pv. *syringae*, but also slightly more susceptible to pathogens, *P.*